Z. klin. Chem. u. klin. Biochem.
10. Jg. 1972, S. 74—76

Serum Cytosolic/Mitochondrial Enzyme Ratio: A Tool for the Estimation of the Severity of Acute Hepatitis

By G. Idéo, R. de Franchis, A. Bellobuono, S. Sforzini and N. Dioguardi

Istituto di Patologia Medica dell 'Universita' di Milano
"Antonio Migliavacca" Centre for Liver Diseases

(Eingegangen am 15. Juli 1971)

The serum levels of the mitochondrial enzymes[1]), aspartate transaminase II, malate dehydrogenase II and glutamate dehydrogenase were studied in 82 cases of acute hepatitis. The activity ratio alanine transaminase/glutamate dehydrogenase and the C/M-ratios of aspartate transaminase and malate dehydrogenase were also studied.
The serum levels of the mitochondrial enzymes glutamate dehydrogenase, aspartate transaminase II and malate dehydrogenase II were "per se" useless in defining the severity of acute hepatitis. Conversely, the activity ratio alanine transaminase/glutamate dehydrogenase and the C/M-ratio[2]) of aspartate transaminase proved to be very helpful for this purpose.
These cytoplasmic/mitochondrial (C/M) ratios were very high in patients who later made a good recovery, while they were surprisingly low in those cases which progressed toward acute liver atrophy.

Es wird die Bedeutung der Bestimmung mitochondrialer Enzyme im Serum und des Quotienten aus cytoplasmatischer und mitochondrialer Aktivität (C/M-Quotient) für die Beurteilung der akuten Hepatitis in Untersuchungen an 82 Patienten gezeigt.
In unserem Material waren die Aktivitäten der mitochondrialen Glutamatdehydrogenase, Aspartattransaminase II und Malatdehydrogenase II für die Beurteilung der akuten Hepatitis nicht bezeichnend, jedoch haben sich die Quotienten Alanintransaminase/Glutamatdehydrogenase sowie Aspartattransaminase I/Aspartattransaminase II als sehr nützlich erwiesen.
Die Quotienten cytoplasmatisches/mitochondriales Enzym (C/M-Quotienten) waren sehr hoch bei Patienten, die sich der Genesung näherten, während sie sich bei Fällen von akuter Leberdystrophie als sehr niedrig erwiesen.

The estimation of the activity of transaminases and other enzymes in the serum, such as sorbitol dehydrogenase, malate dehydrogenase, guanine deaminase etc., is useless for determining the severity and prognosis of acute hepatitis: the prognosis may be good with either low or very high values of serum transaminases; while the same enzymes can fall to low levels in the presence of early liver atrophy. (For reviews on this subject see (1—4)).

Working on the hypothesis that the level of mitochondrial enzymes in serum might correlate with the severity of liver damage in the course of acute hepatitis (5—8), we studied three such enzymes, glutamate dehydrogenase, mitochondrial aspartate aminotransferase, and malate dehydrogenase, in all patients who were admitted to our department with acute hepatitis in the last seven years.

In addition, the activity ratio alanine transaminase/glutamate dehydrogenase and the C/M-ratios of aspartate transaminase and malate dehydrogenase were also estimated in all patients. The activity ratio alamine transaminase/glutamate dehydrogenase is already known to be useful in the differential diagnosis of acute hepatitis and cholestatic jaundice (9—10).

Material and Methods

Anion-exchanger column chromatography was used to separate the mitochondrial isoenzymes of aspartate aminotransferase II and malate dehydrogenase II. We employed columns (diameter 0.9 cm, length 15 cm) with a top feeding funnel, and the absorbent was DEAE Sephadex A 50 Medium. The ion-exchanger resin (activated according to the directions of the Pharmacia Co., Uppsala), was poured into the columns, at room temperature, until the final height of the settled suspension was 7—8 cm. It was then repeatedly washed with Na phosphate buffer, 0.008M, pH 7. The fresh sera, free from haemolysis, were dialysed for 4 hrs at 0—4° in a continuous-flow apparatus, against 25 l of sodium phosphate buffer 0.008M, pH 7. After dialysis, 1 ml of serum was applied to the column at room temperature. After the sample had soaked into the column, elution was performed with 15 ml of sodium phosphate buffer 0.008M, pH 7.
The cytoplasmic isoenzymes of aspartate transaminase and malate dehydrogenase are adsorbed by anion-exchanger resins, such as DEAE Sephadex, while the mitochondrial components are not (11—13). Therefore, only aspartate transaminase II and malate dehydrogenase II are contained in the eluate. The aspartate transaminase I and malate dehydrogenase I activities can either be calculated by subtraction of the mitochondrial activity from total aspartate transaminase and malate dehydrogenase activities of the

[1]) Enzymes: Aspartate transaminase = L-Aspartate: 2-oxoglutarate aminotransferase (EC 2.6.1.1); Aspartate transaminase I = Cytosolic aspartate transaminase; Aspartate transaminase II = mitochondrial aspartate transaminase; Glutamate dehydrogenase = L-Glutamate: NAD oxidoreductase (deaminating) (EC 1.4.1.2); Guanine deaminase = Guanine aminohydrolase (EC 3.5.4.3); Alanine transaminase = L-Alanine: 2-oxoglutarate aminotransferase (EC 2.6.1.2); Malate dehydrogenase = L-Malate: NAD oxidoreductase (EC 1.1.1.37); Malate dehydrogenase I = Cytosolic malate dehydrogenase; Malate dehydrogenase II = Mitochondrial malate dehydrogenase; Sorbitol dehydrogenase = L-Iditol: NAD oxidoreductase (EC 1.1.1.14).
[2]) Abbreviation: C/M-ratio = Cytoplasmic/Mitochondrial ratio.

dialysed sample, or eluted with 15 ml of sodium phosphate buffer 0.2M, pH 7 + NaCl 0.2M. The sensitivity, selectivity and reproducibility of this technique have been repeatedly tested; the results have been reported elsewhere (11). This method has proved suitable for experimental as well as clinical purposes (12—15).

Aspartate transaminase and malate dehydrogenase activities were assayed in serum, before and after dialysis, and in the eluates. Aspartate transaminase activity was determined by KARMEN's method, modified by BERGMEYER and BERNT (16); malate dehydrogenase by BERGMEYER's method (17); alanine transaminase and glutamate dehydrogenase activities were determined in serum by BERGMEYER's and SCHMIDT's methods respectively (18, 19).

Case material

The case material includes 82 patients with acute hepatitis; 70 of them made a good recovery, while 12 progressed toward acute liver atrophy.

Data concerning the former group of patients consist of enzyme values found during the first week after the onset of jaundice; (patients who came under our observation after the first week of jaundice were not included in the case material). As far as the latter group is concerned, the data reported here concern blood samples taken before the onset of coma. Repeated assays of aspartate aminotransferase isoenzymes and glutamate dehydrogenase were made on some of these patients.

The diagnosis of acute hepatitis was based upon epidemiological, clinical, biochemical and histological criteria; the diagnosis of acute liver atrophy was based upon the clinical picture and, in most cases, on the histological features of liver biopsies obtained during necropsy.

Results and Discussion

The mean serum values ± one standard error of aspartate transaminase II, malate dehydrogenase II and glutamate dehydrogenase in acute hepatitis and acute liver atrophy are summarized in table 1; the single enzyme values are reported in figure 1. Although some differences exist, the distribution of the enzyme values was surprisingly similar in the two groups of patients.

The shaded areas of figure 1 indicate the normal range of serum enzyme values, e. g. for mitochondrial aspartate aminotransferase 1.10—4.90 U/l, for mitochondrial

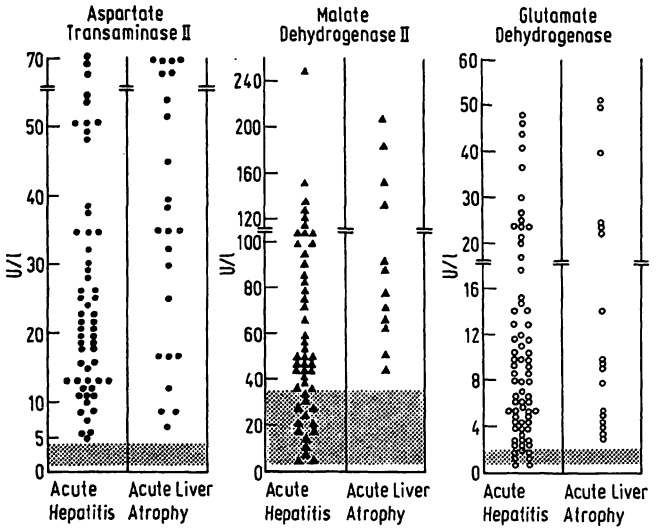

Fig. 1
Behaviour of serum aspartate transaminase II, malate dehydrogenase III and glutamate dehydrogenase in actute hepatitis and acute liver atrophy
The shaded areas indicate the normal range of enzyme values

malate dehydrogenase 6—38 U/l and for glutamate dehydrogenase 0.4—1.3 U/l, whereas the normal ranges of cytoplasmatic enzymes in serum are as follows: aspartate transaminase 4—15 U/l, malate dehydrogenase 34—75 U/l, alanine transaminase 3—18 U/l.

Table 2 reports the mean values ± one standard error of the C/M-ratios of aspartate transaminase and malate dehydrogenase and the activity ratio alanine transaminase/glutamate dehydrogenase in patients with acute hepatitis and acute liver atrophy. The single values for these cytosolic/mitochondrial (C/M) ratios are reported in figure 2. The C/M-ratio of aspartate transaminase was high in patients who later made a good recovery (94.7% of values over 5), and low in those who progressed toward acute liver atrophy (82% of values under 5). The activity ratio alanine transaminase/glutamate dehydrogenase showed a similar behaviour (87.5% of

Tab. 1
Behaviour of serum aspartate transaminase II, malate dehydrogenase II und glutamate dehydrogenase in acute hepatitis and acute liver atrophy
Enzyme activities expressed as U/l

	Acute hepatitis*			Acute liver atrophy**			
	N	Mean	S. E.	N	Mean	S. E.	P
Aspartate transaminase II	56	26.09	±2.26	24	38.04	± 4.47	<0.02
Malate dehydrogenase II	49	64.02	±6.68	12	103.78	±13.39	<0.01
Glutamate dehydrogenase	70	11.62	±1.3	17	17.1	± 3.9	<0.05

* First week after the onset of jaundice.
** Before the onset of coma.

Tab. 2
C/M-ratios of aspartate transaminase and malate dehydrogenase and activity ratio alanine transaminase (GPT)/glutamate dehydrogenase (GLDH) in acute hepatitis and acute liver atrophy

	Acute hepatitis*			Acute liver atrophy**			
Ratio	N	Mean	S. E.	N	Mean	S. E.	P
Aspartate transaminase I/II	56	14.31	±1.321	22	4.52	±0.85	<0.001
Malate dehydrogenase I/II	49	3.54	±0.776	12	0.99	±0.08	>0.05
GPT/GLDH	48	54.04	±5.53	12	7.5	±1.72	<0.001

* First week after the onset of jaundice.
** Before the onset of coma.

values over 16 in the former group of patients; 92% under 16 in the latter one). The C/M-ratio of malate dehydrogenase showed no differences in the two groups of patients.

As far as the aspartate transaminase isoenzymes are concerned, our results are in agreement with that of VILLA and coworkers (20), who observed a significant increase in the per cent aspartate transaminase II activity in the course of acute liver atrophy.

We believe therefore that the estimation of the serum levels of mitochondrial enzymes alone is of little value in predicting the development of acute hepatitis: but the estimation of the C/M-ratio of aspartate transaminase and the activity ratio alanine transaminase/glutamate dehydrogenase can be very helpful for the early diagnosis of acute liver atrophy. This is very important to the physician who has to consider the more heroic forms of therapy for liver failure — such as steroids in high dosage, exchange transfusion, extracorporal liver perfusion or liver transplantation.

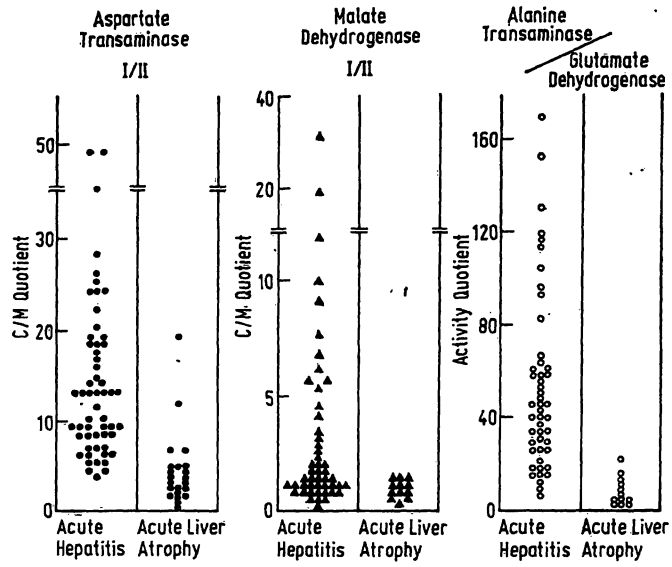

Fig. 2
Serum aspartate transaminase and malate dehydrogenase C/M-ratios and alanine transaminase/glutamate dehydrogenase activity ratio in acute hepatitis and acute liver atrophy

References

1. ZIMMERMAN, H. J., Gastroenterology, *46*, 613 (1964). — 2. SCHMIDT, E. and F. W. SCHMIDT in BERGMEYER, H. U. "Methoden der enzymatischen Analyse" vol. I p. 14, Verlag Chemie. Weinheim/Bergstr. (1970). — 3. COODLEY, E. L., Amer. J. Gastroenterol., *52*, 189 (1969). — 4. ZIMMERMAN, H. J. and L. B. SEEFF in COODLEY, E. L. "Diagnostic Enzymology" Ed. Lea & Febiger, Philadelphia, Pennsylvania, *1*, 38 (1970). — 5. FLEISHER, G. A., D. C. S. POTTER and K. G. WAKIM, Proc. Soc. exper. Biol. Med., N. Y. *103*, 229 (1960). — 6. SCHMIDT, E. and F. W. SCHMIDT, Enzymol. Biol. Clin., *3*, 1 (1963). — 7. SCHMIDT, E., F. W. SCHMIDT and P. OTTO, Clin. Chim. Acta, Amsterdam *15*, 283 (1967). — 8. SCHMIDT, E., F. W. SCHMIDT and J. MOHR, Clin. Chim. Acta, Amsterdam *15*, 337 (1967). — 9. FILIPPA, G., Enzymol. Biol. Clin. *3*, 97 (1963). — 10. RETTENBACHER-DÄUBNER, H., H. RIEDER and I. SBURNY, Acta hepato-splen. *14*, 209 (1967). — 11. IDÉO, G., R. DE FRANCHIS, E. DEL NINNO, A. BELLOBUONO and G. FIORELLI, J. Chromatog., *52*, 162 (1970). — 12. IDÉO, G., R. DE FRANCHIS and E. DEL NINNO, Experientia, Basel *26*, 487 (1970). — 13. IDÉO, G., R. DE FRANCHIS and E. DEL NINNO, Enzyme *12*, 242 (1971). — 14. IDÉO, G. and R. DE FRANCHIS, this Journal *8*, 611 (1970). — 15. IDÉO, G., R. DE FRANCHIS, A. BELLOBUONO, E. DEL NINNO, Enzyme, in press. — 16. BERGMEYER, H. U. and E. BERNT, in Bergmeyer, H. U. "Methods of enzymatic Analysis", Academic Press, New York, p. 837 (1963). — 17. BERGMEYER, H. U., E. BERNT and B. HESS, in Bergmeyer, H. U. "Methods of enzymatic Analysis", Academic Press, New York, p. 757 (1963). — 18. BERGMEYER, H. U., E. BERNT and B. HESS, in Bergmeyer, H. U., "Methods of enzymatic Analysis", Academic Press, New York, p. 846 (1963). — 19. SCHMIDT, E., in Bergmeyer, H. U., "Methods of Enzymatic Analysis", Academic Press, New York, p. 752 (1963). — 20. VILLA, L., N. DIOGUARDI and A. AGOSTONI, Klin. Wochenschr. *45*, 44 (1967).

Prof. Dr. G. Idéo
Corso di Porta Romana 106
I-20122 Milano, Italy